

Cloning and characterisation of a gene encoding the 11.5 kDa zinc-binding protein (parathymosin- α)

Hans-Ingo Trompeter and Hans-Dieter Söling

Abteilung Klinische Biochemie, Zentrum Innere Medizin, Universität Göttingen, Robert-Koch-Str. 40, D-3400 Göttingen, Germany

A cDNA clone encoding the 11.5 kDa zinc-binding protein (ZnBP) was used to screen a genomic and a subgenomic rat liver library to isolate the corresponding genomic DNA. Positive clones were restriction-mapped and sequenced to give the primary structure of the ZnBP gene. A putative promoter region was detected.

Zinc-binding protein, 11.5 kDa; Parathymosin- α ; Genomic DNA; Subgenomic library

1. INTRODUCTION

The 11.5 kDa zinc-binding protein (ZnBP) was first described by Brand and Söling [1,2] as being capable of inactivating phosphofructokinase-1 (PFK-1; EC 2.7.1.11) in a Zn^{2+} -dependent but reversible manner. Affinity chromatography revealed its ability to also bind to other glycolytic and gluconeogenic enzymes in the presence of Zn^{2+} [3]. ZnBP is found in the cytoplasm of liver, brain, adrenal gland, smooth muscle, kidney, lung, spleen and testis, whereas it is only weakly detectable in skeletal muscle and adipose tissues [4]. Cloning and sequencing of its cDNA [5] revealed the identity of ZnBP with rat parathymosin- α [6,7]. One interesting aspect of the primary structure of ZnBP is that it contains in its C-terminal region a sequence (PKRQKT) resembling the prothymosin- α nuclear targeting signal [8].

We have now cloned the genomic DNA encoding the ZnBP transcript in order to get more information about regulation of expression of ZnBP and to possibly detect isoforms.

2. MATERIALS AND METHODS

2.1. Materials

Restriction enzymes were purchased from Gibco/Eggenstein (Germany), T4-ligase, Hybond-N filters, [α - ^{32}P]dCTP and [α - ^{32}S]dATP were from Amersham-Buchler/Braunschweig (Germany). Calf intestine alkaline phosphatase (CIP) was obtained from Boeh-

ringer/Mannheim (Germany); proteinase K, DNase I and RNase A from Sigma/München (Germany). M13mp18/19RF-DNA was from Pharmacia/Freiburg (Germany), gt10-DNA, the Gigapack II Plus packaging extracts and the lambda DASH rat genomic library came from Stratagene/Heidelberg (Germany). The EMBL-3 rat liver genomic library was from Clontech-Renner/Dannstadt (Germany). GeneScreenPlus membranes came from NEN-Dupont/Bad Homburg (Germany), autoradiography was performed using Kodak XAR-5 films. Male Wistar rats came from Winkelbach/Dernbach (Germany).

2.2. Methods

Lambda-DNA was prepared from plate lysates, digested with the appropriate restriction enzymes and analysed on 0.4%–0.8% agarose gels. *EcoRI* insert DNA from cDNA clone H9 (H9I) [5] was eluted out of the gel and labelled with ^{32}P using the random priming method [9]. Hybridization to DNA bound to Hybond-N- or GeneScreenPlus-filters was performed as in [10].

Preparation of genomic DNA from rat liver. A fresh rat liver was washed in fresh, ice-cold sucrose buffer (0.3 M sucrose, 60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.05 mM spermidine, 15 mM HEPES, 2 mM EDTA, pH 8.1, and 0.5 mM EGTA) and homogenized in 60 ml of this buffer. The homogenate was centrifuged at 1000 \times g. The pellet containing the nuclei was washed twice with the above sucrose buffer and resuspended in 60 ml of TNE (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, 5 mM EDTA, pH 8.1). 1.5 ml of 20% (w/v) SDS and 1.5 ml of proteinase K (2 mg/ml in TNE) were added and the mixture was incubated overnight at 37°C with gentle shaking. The viscous solution was carefully extracted with phenol/chloroform/isoamylalcohol (25:25:1, v/v/v) and precipitated with ethanol. DNA was resuspended in 50 ml of 0.1 \times SSC (15 mM NaCl, 1.5 mM Na-citrate) and dialysed against 0.1 \times SSC. After treatment with RNase A (100 μl of 10 mg RNase A/ml 10 mM Tris-HCl, pH 7.5, 15 mM NaCl, 1 h, 37°C), the DNA was reincubated with 250 μl of proteinase K solution (1 h, 37°C), three times extracted with phenol/chloroform/isoamylalcohol, precipitated with ethanol and resuspended in 50 ml of TE-buffer. After dialysis against TE for three hours the DNA was analyzed on 0.2% agarose gels.

Construction of a subgenomic library. Genomic DNA from rat liver was digested with *BamHI*, *EcoRI* and *HindIII* and the resulting fragments were alkali-blotted onto GeneScreenPlus membranes and hybridized to H9I.

A region between 5 and 7 kb of *EcoRI*-fragments was eluted out of an agarose gel, dephosphorylated with CIP, and ligated to 1.1 μg of *EcoRI*-cut gt10-DNA in a molar ratio insert-vector of 2:1. The ligation

Abbreviations: bp, base pairs; CIP, calf intestine alkaline phosphatase; H9I, insert-DNA of cDNA clone H9; kb, kilobase pairs; ZnBP, 11.5 kDa zinc-binding protein.

Correspondence address: H.-I. Trompeter, Abteilung Klinische Biochemie, Zentrum Innere Medizin, Universität Göttingen, Robert-Koch-Str. 40, D-3400 Göttingen, Germany.

reaction was in-vitro-packaged using the Gigapack II Plus extracts and the resulting library was titered on *E. coli* C600hflA.

Library screening and clone sequencing. The gt10-phages were plated onto *E. coli* C600hflA, the EMBL-3 library on *E. coli* K803 and the lambda DASH library on *E. coli* P2PLK17. Bacteriophage plaques from the libraries were transferred onto Hybond-N membranes according to [11] and hybridized to insert DNA of clone H9. Positive plaques were picked and single positive plaques were obtained by two rescreening cycles using the same probe. Phage DNA of genomic clones was restriction-mapped with *Bam*HI, *Eco*RI, *Hind*III, *Kpn*I, *Sac*I, *Sst*I and *Xho*I.

Fragments chosen for sequencing were eluted out of a gel and subcloned into M13mp18/19, insert-orientations being examined according to [11]. The fragments were sequenced using the Sequenase and Taqenase kits according to the manufacturers instructions using parallel dGTP and dTTP runs respectively.

3. RESULTS

Earlier studies had led to the isolation of gt11-cDNA clone H9, which carries a 936 bp *Eco*RI insert (H9I) representing the complete translated region of the ZnBP [5], 115 bp of 5'-nontranslated region and the complete 3'-nontranslated region including a poly(A) tail. It displays a *Bam*HI restriction site at base 384 only 11 amino acid residues upstream the C-terminus of the encoded protein. H9I was used as a highly specific probe in Southern analyses of rat genomic DNA. One *Eco*RI fragment (about 5.5–6 kb), one major *Bam*HI fragment (about 1 kb) and a weakly hybridizing *Bam*HI fragment (about 2.9 kb) were detected. These results indicate, that H9I is transcribed only from a single-copy gene.

To isolate the genomic DNA corresponding to H9I, 2×10^6 clones of a rat liver genomic EMBL-3 library were screened using H9I as a probe but no positive clones could be isolated. To ensure isolation of the gene encoding the H9I transcript, a subgenomic library of 5–7 kb *Eco*RI fragments in lambda gt10 was constructed and a lambda DASH rat liver genomic library was screened additionally. 5×10^5 gt10 clones and 10^6 lambda DASH clones were screened using 32 P-labelled H9I as a probe. Three subgenomic gt10- and two genomic lambda DASH-clones (H9D1 and H9D2) were isolated during two rescreening cycles and subsequently analysed by Southern blotting and restriction mapping. All subgenomic gt10 clones carried a 5.7 kb *Eco*RI insert hybridizing with H9I. *Sac*I-inserts of lambda-DASH clones were about 18 kb long and cross-hybridized with H9I and the 5.7 kb *Eco*RI subgenomic fragment as well. Subsequent analyses of the lambda DASH clones with *Bam*HI, *Eco*RI, *Hind*III, *Kpn*I, *Sac*I, *Sst*I and *Xho*I yielded a restriction map of the genomic region encoding the H9I-transcript (Fig. 1). A 5.7 kb *Eco*RI fragment hybridizing to the subgenomic 5.7 kb fragment was found as well as 1.0 kb and 2.9 kb *Bam*HI fragments. These results are in line with the above Southern analyses of genomic DNA. The 5.7 kb *Eco*RI fragment found in the subgenomic gt10 clones displayed the same *Bam*HI pattern as the 5.7 kb *Eco*RI fragment

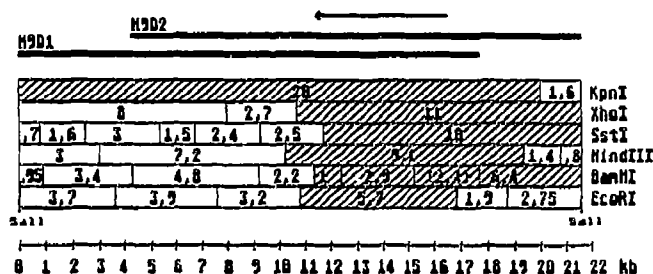


Fig. 1. Restriction map of the genomic clones. The region of DNA spanned by *Sac*I inserts of clones H9D1 and H9D2 is shown. The black bars show the position of the two clones, the arrow marks the direction of transcription. Fragments hybridizing with H9I are hatched, the 2.4 kb *Bam*HI-*Sac*I border fragment of H9D1 is marked by the interrupted vertical line in the *Bam*HI row. The position of the 1.9 kb and 2.75 kb *Eco*RI fragments is arbitrary. The lengths of fragments are given in kb.

of clones H9D1 and H9D2 and was thereby shown to represent the same DNA. The 2.9 kb *Bam*HI fragment hybridized to the 5'-*Bam*HI fragment of H9I, the 1.0 kb fragment to the 3'-one, indicating the direction of transcription.

Since both libraries yielded clones representing the same region of genomic DNA, the 1.0 kb and 2.9 kb *Bam*HI fragments, a 2.4 kb *Bam*HI-*Sac*I fragment (represented as a 6.4 kb *Bam*HI-*Sac*I border fragment in H9D2) and the 5.7 kb *Eco*RI fragment (to verify the borders between the short fragments) of H9D1 were subcloned into M13mp18 and M13mp19 and sequenced. The complete sequence of 6301 bp is shown in Fig. 2.

4. DISCUSSION

Since intensive screening of an EMBL-3 rat genomic library yielded no positive clones, a subgenomic library based on genomic Southern analyses was constructed in order to overcome possible cloning restrictions resulting from e.g. unstatistical distribution of *Sau*III sites on the genomic DNA of ZnBP, thus leading to a low representation of this gene in a genomic library. The subgenomic library as well as an additionally screened lambda-DASH rat genomic library yielded several clones. Restriction analyses and cross-hybridizations between both clone families showed them to represent the same DNA and were in line with the Southern analyses of genomic DNA.

The genomic fragments hybridizing with H9I spanned a region of 6301 bp and contained the whole transcribed region represented by H9I with identical corresponding sequences (see Fig. 2). The translated part (printed bold in Fig. 2) is interrupted by one large (2589 bp) and three small (191 bp, 150 bp and 167 bp) introns. No introns were detected in the regions corresponding to the nontranslated parts of H9I.

A possible promoter region was detected in a region

GTCTGCTAAT	ACGACTCACT	ATAGGGCGAG	AATTCGGATC	AAGAATCCCT	50	AAGGCAGTGG	GAGAGGGGAC	ATGTGAGATC	TGATAAGGTG	TGCTGTGTGT	2950
TCCTCTCTCT	TTTCTTTTCT	AATTTTTTCT	GAGGGGTAGG	GGAGGGCAAG	100	GCCTCTCCGT	GGGTGTGTGG	GTGGCATGTG	ACTTCACTGA	GTAGGGCTCT	3000
CTATCTCACT	GTGTGTGCTA	GGATGCTCTC	AAACTAATCC	CAATCTCTCT	150	TGTATGTGCT	GAGATCCAGC	GAGTGTGTGG	GACTGCCGCT	GACTGTTACA	3050
GCCTCAGCTT	CCCAAGTGCT	AAATAGGTCA	TTTGACTAAA	GGTGATGGTT	200	TGTGTGATG	TGGAGACTTA	CTGAGATGTC	CTGGAGGACT	GTTCGTGCTT	3100
TTTCTGTGTT	GTCTTTTCTT	CGGAGCTGGG	GACCGAACCC	AGGGCTCTGC	250	TGTAAGTATC	GGGTGGCTAC	ACTGTGCTTC	TGAGAGTGTG	CCAGAAATGC	3150
TAGGGCCTTG	CTCTTGCTAG	GCAAGCGCTC	TACCACTGAG	CTAAATCCCC	300	CAAACTATTG	GGCATGTGTC	TCCAGAGTGT	GTGGGGCTCT	CCTTGCAGAT	3200
AACCCCTAAA	CAACCTGTAA	ATGTGCAGAG	AGGAATCTCT	CTCTCTCTTT	350	GTGTGAGTTG	GTGTTAGCTC	GGGTGAGCTT	GTGTACCCCT	GTAGAGGCCA	3250
TTTCTTTTCT	TTTCTTTTCT	TGGGAGCTGG	GGACCGAAC	CAGGGCTCTG	400	GAGAGCTACT	AAGCAGCTGG	GGGCAGGTCA	CATACCTCTC	TGGAAGAGAG	3300
TGCTCGCTAG	GCAAGCGCTC	TACGGCTGAG	CTAAATCCCC	AACCCCTAAG	450	ATCATGGGCA	GACAGTGACC	AGGACAGGAC	CAGGAGGAG	AGGGGCCAAC	3350
ATGATGGTTT	TTTATTATGG	AAATAAAAAT	GATTAGAGAA	AGGTGAGGGA	500	CTGTGGACT	TTGATCTCTG	GTATTCGCC	TCCCTCTCCC	TCACCCCTTC	3400
TGATGATGAC	AGATAAGACA	CATACACCAA	AGACAGTCTT	TTCCCAAAGC	550	ATCAACCCCA	ATAGAAAAC	CAGGCTTGCA	AGGCTGGAT	CAGGCTAAGT	3450
CCAGACTTCT	TCATCCACCC	TTGGAGGCGG	TCGCTTTCTG	AAACCCGGGG	600	TGACTTAGTC	CTGCTCTGGC	CGGGATGTA	AGAGAAAGCT	CACCTGGTGC	3500
TTCTCAAAAT	GATCTAAAAG	TGCTGTACCT	AGGTGTGAAG	CCCTGAGGAT	650	TTCAAGTGCC	AGACCTACCA	TGTGACCGAG	AAGTACAGAA	TCAGAATGAG	3550
AAGCGCAATA	TGGGTGTGGC	TGGAACCCCA	TATCTGGACC	AGTGTGTGCA	700	GAAACCTGCC	TGGTGTGAAA	GGGTCAAGTG	TTTTGTGCTC	TTCTCTGAAT	3600
AAGCGCGCCA	GTGTAATCTA	GAAAGACTGG	GGGCAAGATA	GAAGCAGAAG	750	GAGAAGGGAG	TGGAGTGTCC	ACAGGCCAGT	CATTCTCGAC	CTCTCTTACC	3650
ACCGAGAAGA	GCGACTGGAG	GAAATAATGA	AAAGGACAAG	AAAGAGAGTT	800	TCACCTCTCT	TCTAGAAAGG	ACACATCTCT	CTGGGGTGAT	CTCTCTATGA	3700
GAAATTTAGC	TAAGTTTATA	GGGCGGGTGG	ACTGAGTCAG	GAAAGATAAC	850	AAACTCCGAC	CGAATTTGGG	ATCATCATCT	CAAGAGTTGC	ATGCTAACAT	3750
AGTGTGTCTC	GGGAAAAAGT	AAATTTGAGC	ACATGGGATG	TAGATGGTTA	900	TAGATAAGGT	CTAGGACAAA	GCTCGCTCTC	GTCTCTCTCC	CCCCCCCCCA	3800
TAAGAGTAGG	GCTCTGGTCT	TATGGAGAGG	GGGCGAAGGG	AAACCAAGGG	950	CACACACACA	CCAAGGACAA	GCAGAACTCT	CATCGGCTAG	GAAGGCTGGT	3850
ACCAAGcaat	TGAGTGAGGG	GAGCGGGTGA	AGATGAGAGA	AGaaataaag	1000	GTGTCCAGAG	GGGCTTACCC	ACTGGCCCAA	TAGTATAGGA	CCTCAAGCAG	3900
GGCAGGAAGG	ACCATCAGAG	CGCTCTCACA	TACACAGAAA	CAGACAGAAA	1050	CTCGCCAGGA	GGCAGACAGT	CTGGTGGGAG	GGGCTCTGGA	CAGGTAATCT	3950
GGAAACCCCG	AAGGACCACA	AATGTCAAGT	CCAGCCGAGA	GGCCAGGGCT	1100	CACACTTCCC	TCCCGGCCGC	ACACTTTGTT	TTTGTGCTTT	TTCTCTCTCT	4000
GTCTTTTGTA	CTCTCACTTT	CTCTCTCAAG	GCCACTAGAG	GAAAGCGGAC	1150	TGCTTGAAGG	TGGGATGTGC	ACAGGGAAGC	CTGAGGCCGT	TTAGTATGGG	4050
AAGAGCGAGA	AATGGAAGTG	GCTCAGGAGG	CTTCTCTCTC	TTCTCTCAGC	1200	GATAGAAACC	GATGTGGGAA	ACGTTAGAGA	AGTATCGGCG	CAGATAGGAG	4100
TTTTGTTTTC	TCAAATACCA	TACTATGCTC	TACTATGCTC	CCGCCACCCA	1250	TAGGAGGATG	AGGGAGACAG	AAACGAAAC	AAAGACCTTA	GGAGCTGAAG	4150
CCGCCCGCTT	TGCAAAAAAT	TGAGGCTCTT	GAGGAGGTCA	TCGAGAGCTG	1300	AGGAAGCTGG	GAGGATGACC	CAGGAGAGAG	CAAGCTTAGG	CAGAGGCCCC	4200
GGCCCACTCT	TCAGTCCGCC	TCAGTCCGCC	AGACTCCAGC	TCCGAGCGAG	1350	TGAGGTAGAC	AAAGCCGAGA	CGGGGGCAAG	CAGAGAGGGT	GAGGGGGTGG	4250
AAGGCTGGCA	TTTGAGGCGG	TCTCTCCCGG	CAGGAACCCC	ACCCCTTCCC	1400	GGCAACACAG	GGCTCAGCAG	ATGGGGGATT	TGCTTTGTGG	AGCTGAACCT	4300
ACCTCTCTGC	CTACTCTGCG	CTCTTCCCGA	CGCGTCTCTC	CTCGCCCTTC	1450	TTAAGCCAGG	GGATTTTGGG	AGAACTTCTA	TGGGGTGGGA	GGCCCAACTA	4350
CCCTCCCGGT	CTCGTCTCTC	CTCGTCTCTC	CTCGTCTCTC	ATCCCTCTCT	1500	CTCTGGCCAC	TTATCATCAC	TTGCCCACTG	CTCTTGGCTC	TTGGGGACAA	4400
CTTTCATGCT	CTCTATCCCA	CTCTCTCAAC	CTCTCTCTTC	ATCCCTCTCT	1550	AAATCTCTGG	GATCAGCCAG	TTATGGCCCA	CAATTAAGAA	TCCAGGAGCT	4450
CTCTCTCTCT	CTCTCCCGCT	CTCTCCCGCT	CACCCCTCTG	GAAAGCCCTC	1600	CTCTGTTTGG	TTCACTCAAT	GGCCACCTCT	AGATTAACCT	TTTGACCTAG	4500
CGCTGGCGAG	CGCCGCTTCA	AAAGCGGCTT	CGCCGCGGAG	CAAGACCGAG	1650	CTCGAGAGTT	GAAAGGACTC	ACCCCTGTCT	GTACCCCTCT	CTGAGAGAGA	4550
GGCTGGTGGG	CGGAGCTTCT	GCTGTGCTGC	GGGCGGCGAG	CAAGACCGAG	1700	ACCTTCTCTC	TGAGGAGACC	TGAAGGAAAG	GAAAGGCAAG	GTGGAGGAGA	4600
CGACCGGAC	CGGAGCTTGC	CGCCGACCCG	CGGATCATCT	CTCTCTCCCT	1750	AGGGTGGGCG	GAAAGAACCG	AGAAAGAAAG	TAGTGGAGGT	GTGTGTGAAG	4650
CCCTCCGAC	GGCGCGAGCC	CTCGGGGTCT	CGCTCCCGGA	CCACCCCGCC	1800	CGAGAATATG	AAACACATTT	GGCCAAACCT	CCTCTTCTCT	TACCTCTCTG	4700
CCCCACCCCG	CGCGCTCTTG	CGCGCTCTTG	CTGCGACCCA	GCTTCCCGAG	1850	GGATGACCTC	CTGCCATTTT	CTTGGCCCTT	TCGTATCCCT	TCCTCTGCTG	4750
CGCGCTCTGC	TGCGGTCTCG	CTCGGCTCTG	CGCCACCGCG	CCAAAGTTCC	1900	CGCCCTCTCT	AGCCCTTTTA	CCACTCAGTG	CCCTTCTCTC	CAGCCTGCTT	4800
CGCGCGGACA	CGCTCCGCGC	TCGAGGCTCT	CTCGGCTCTG	CGCCCGGAGC	1950	GACATCACTC	CTTGTTTTGG	TCCCCACAGG	AGGAGGAGAA	TGGAGCTGAG	4850
CGCGGCTCCC	CGCCAGCCCC	GGCCCGGCGA	CAATCTCGGA	GAAAGAGCTG	2000	GAGGGGAGAA	GAAAGAACTG	AGAGGATGGA	GAGGATGATG	ATGAAGGAGA	4900
CGGAGCAGGG	CGGAGCTAAG	CGCCAAAGTA	CGGCGAGGGG	CGGCGGCGCG	2050	CGAAGAAAGT	AGGGATGGGG	AGGGCCGGCT	GGCTTGCAAA	TTTGAGACTC	4950
CGGAGACCCG	CGGAGCGGCG	GGATCGGCGC	AGCTCTTGCG	CCCGGTCCGC	2100	ACAGGAGCAG	AGTCAAGGAG	CAGTTGAAGA	CTTGAAGGCT	GGTATGAGGG	5000
CGGAGCGGCG	CGGAGCGGCG	CTGCGCGGCG	GTCCCGGCGG	AGCTCGCTGC	2150	AGGGGTCTGT	GGCCCTGCTC	CCCAACCTTC	CGCCATTGAC	TTTGTCTCTG	5050
GGCTCTCCCG	CTTCCCGGCT	CGGAGCGGCG	CGCTCGGCTC	GGTCTCTCTG	2200	TTTCTCAGAT	GAGGAGGAGG	AGGAGGAGAG	CGCCAGAGGG	CCCGTGGCGA	5100
CGGCGCTCTA	CGGCGGCTCT	CGGCGGCTCT	TCGCGGCTCT	CGCCCGGCGC	2250	AGAGAACTGC	TGAAGAGGAG	GTTTGAGTGA	AGGGGCTGTC	GGGAATATAG	5150
TGCGCATCCC	CACCCCTCTC	GTGCGCGGCG	CGCCAGTCTC	GGCCGCGTCC	2300	CAGGGCTGTC	TGACCTTTTG	GATTTGGACC	GATTTGGCTG	TGGGTGCGAT	5200
GGCTCTCCGT	CAGAGAGTGG	CGCCTCTCAG	ATCCCGGCGC	CCATGTCCGA	2350	AGATGGGCG	GGGCTGGAGC	AGGGCCAGCA	GGAACGGGGG	AGGGGGGGCT	5250
ACCGGGGCTC	CAAGTCCCTT	TCCCATATGA	CTCTACCTTT	CTCTGTCTAA	2400	CTCTCTGTAC	ACTACTCAAG	CTCTTCTCCC	TCCACAGGAT	GAAAGCGATC	5300
CGCGTTTGGG	ATCTTCAAGT	GTAACACAGG	CTCTCCCGGG	AGGCTACTCC	2450	CCAAAGAGGA	GAAAGACAGG	AACGGGGCGT	CGGCTTGGG	CCCTGCGGCT	5350
TCGCGTCTCT	CCCTCTCGCG	CGCCAGCGCT	CTCTCTGCTC	CCGAGAGGGG	2500	GGGCTTGGGG	ATGGGAGGCC	CCTCAGGTCC	TGGAGGTGGG	CGAGGAACAC	5400
TGCTCTTCTC	TGCTACCCCG	CACCCCTTTT	TTTTCTTTT	TGCGCATCTT	2550	ACAAATCCAG	CCCCCTTCTT	CTCGGCTCCC	TGCTCTGAGC	CTGCCCCAGA	5450
CATTCTGTCT	TGCTTAATA	CTGACTTCCA	TTTCTCTCAT	TATCGGCTAT	2600	GGTGTGACCC	TGCGCTTTTG	ACCCAGCTCC	TCATTCTTCC	CTCTCCAGAC	5500
CCCATGTGGA	ATTCTAAAA	AATCAGAAAG	GTGGCGAGAA	TAGCTTCCGA	2650	ACTGTCTCTT	CACCTCTACT	GGCCACAGTC	CAGCCCCCAA	CCCGCTCTAC	5550
AAAAGTACAA	GCTCATCATC	TCTGACCAT	ACGTCAAGCT	GTCTTAGCTC	2700	CCAAAGCTCC	CAGCGGGGCC	TCACTTGGCC	TAGCATCTCT	TGTTCTTCCC	5600
TCAGTAGCTC	CTTTTCCCGG	CTTCTCTCTT	TGCGGCTTGT	CTCTCTTTCA	2750	TGCTCTCTCT	ACCATCGATC	TGTTTCCAGT	CTTGGGAAAG	CTCTCTCTCT	5650
GCCTCTCTCT	AATTTCCCCA	GGGAACTTGC	GGAGTGTGCG	TTAGGGAAT	2800	CTCTCTGACC	CCGAGCTCTC	CAGGCTGCCC	TTCTCTCTCC	TGCTGACCCC	5700
TAATCGCGCC	AGGCTTGGGG	GCTGGGAGCC	CAGTCTTTT	TTTGGGGGG	2850	CTGGGTCTCC	CTCAGATTCC	CTCTCTCAG	ACAGGCGCAG	GGCGGGGTGG	5750
GGCACTATAG	TTCTCCAGGT	CAAAGTCCAA	GGGGAGTCT	AATAGAATGA	2900	GGCTGGGGTT	GGGGCCAAGC	CCGGAAGCTG	CCCCCTCCCC	TTTTTGTATA	5800
						ATTTAATAAA	GAAACGGTGG	CGCTTCTGTT	TTTAAACCGT	CTCTGCTTTT	5850
						CCCAAGTCC	AGTCAAGGGA	TGAAAGGGTG	TAGAGGGTGT	GGTTGACCTC	5900
						ATACCTCTGAT	TATAGAAGAC	ATGGCCAGCT	CTTGAGTGGG	AGCTCAAGGA	5950
						CTTCTCTCA	CGGCTGAAGG	GTGTTTGTGG	GGTTCCTAGG	GGTGTCTCTC	6000
						CTCAGCGATG	CACACTATCA	TGTTAACTCA	CGTAAGATCT	AGACACTGCT	6050
						GAGGCCAGTG	GAAAGCTCCA	TTGTGCTGAA	CCAGGCAGTT	AGAAACAGGA	6100
						AGTCTCTGCC	TCAAGGTGGA	AGAGGCTGGT	AGGCTTAGGT	CTCTACCTAG	6150
						CGTTTGGAAA	GAGAACTCCC	AAGCACGGCT	GTGGGAAGCA	GGGTCTCTCT	6200
						TGGCGGAGTA	GCAACAGACT	TCAGAACTAG	TTATCAGACC	TTTCTCTCTG	6250
						TGCTCTGTCT	TCATTTTCTC	AGCTGAGCTG	TGTGTGATC	TTGTTGATC	6300
											6301

Fig. 2. Sequence of the genomic DNA encoding ZnBP. The 6301 bp region of the subcloned fragments is printed in CAPITAL letters, beginning with the *Sa*I site and ending with the *Bam*HI site. Possible promoter regions are printed in small underlined letters. DNA represented in H9I (mainly exons) is printed bold; start- and termination-codon as well as the polyadenylation signal are underlined. The underlined region at the beginning of the sequence marks the 'rat identifier'.

from base 830 to base 1000 (underlined in Fig. 2). The sequence AATAAAG, beginning at base 993 shows strong homology to the consensus sequence of the TATA-box, GCAAT (base 956) a good homology to the CAAT-box. Two possible GC-boxes are also underlined in Fig. 2. This configuration would implicate a possible transcription start around base 1020 which would give a transcript of about 1770 bases (excluding the poly(A) tail), assuming that there are no introns in the 5'-nontranslated region. This value is in good agreement with the finding of a 1800 base transcript for parathyroidin- α by Clinton and colleagues [13].

No hint of a possible isoform of ZnBP synthesized by differential splicing of a premature mRNA was found in any reading frame of the gene. Only one additional

donor site (AGGT; base 1284) could be detected in the 5'-nontranslated region. Therefore a differentially spliced isoform seems unlikely. If at all, a possible isoform should be transcribed from a different gene which could not be detected by H9I under stringent hybridization conditions.

A computer search in the EMBL database/Heidelberg (Germany) showed no relationships of the genomic DNA with other known sequences except for the existence of a 'rat identifier' sequence [14] upstream the promoter region.

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